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Predictors of Inadequate Linezolid Concentrations after Standard Dosing in Critically Ill Patients

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Adequate linezolid blood concentrations have been shown to be associated with an improved clinical outcome. Our goal was to assess new predictors of inadequate linezolid concentrations often observed in critically ill patients. Fifty-two critically ill patients with severe infections receiving standard dosing of linezolid participated in this prospective observational study. Serum samples (median, 32 per patient) were taken on four consecutive days, and total linezolid concentrations were quantified. Covariates influencing linezolid pharmacokinetics were identified by multivariate analysis and a population pharmacokinetic model. Target attainment (area under the concentration-time curve over 12 h [AUC_{12}]/MIC ratio of >50; MIC = 2 mg/liter) was calculated for both the study patients and a simulated independent patient group (n = 67,000). Target attainment was observed for only 36% of the population on both days 1 and 4. Independent covariates related to significant decreases of linezolid concentrations included higher weight, creatinine clearance rates, and fibrinogen and antithrombin concentrations, lower concentrations of lactate, and the presence of acute respiratory distress syndrome (ARDS). Linezolid clearance was increased in ARDS patients (by 82%) and in patients with elevated fibrinogen or decreased lactate concentrations. In simulated patients, most covariates, including fibrinogen and lactate concentrations and weight, showed quantitatively minor effects on target attainment (difference of ≤9% between the first and fourth quartiles of the respective parameters). In contrast, the presence of ARDS had the strongest influence, with only ≤6% of simulated patients reaching this target. In conclusion, the presence of ARDS was identified as a new and strong predictor of insufficient linezolid concentrations, which might cause treatment failure. Insufficient concentrations might also be a major problem in patients with combined alterations of other covariate parameters. (This study has been registered at ClinicalTrials.gov under registration number NCT01793012.)

With mortality rates ranging from 15 to 50% (1–5), severe infections are among the most prevalent causes of death in intensive care unit (ICU) patients. Therefore, it is of high clinical relevance to establish proper treatment strategies leading to adequate blood concentrations of antibiotics in order to maximize the effectiveness of treatment (6), limit adverse reactions (7), and prevent the development of antimicrobial resistance.

Linezolid is an important antibiotic agent for severe infections and has a good antimicrobial activity against Gram-positive strains, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. The plasma elimination half-life is 3.1 to 4.9 h, with a clearance rate of 6.4 to 14.8 liters/h (8) and a protein-bound fraction of about 31% (9). The primary metabolic pathway is the oxidation of the morpholine ring, resulting in an aminoethoxyacetic acid metabolite and a hydroxyethyl glycine metabolite (major metabolite) (10). The latter substance results from the lactone pathway, where the initial oxidation step is chemical rather than enzymatic (10). Reactive oxygen species are assumed to play an important role in this pathway (11). About 30% of linezolid is eliminated unchanged by the kidney (12).

Standard dosing of 600 mg linezolid twice daily (b.i.d.) is recommended for all adult patients by the product information and has been included in treatment guidelines. However, recent studies have observed a high variability of linezolid blood concentrations, with partly insufficient concentrations in critically ill patients treated by this standard scheme (13–17). We recently observed that 19 of 30 critically ill patients had insufficient concentrations and that 2 of them reached potentially toxic serum concentrations (13).

There may be numerous reasons for the observed high variability of linezolid concentrations in critically ill patients. Several predictors, such as glomerular filtration rate (14, 18–22), body weight (18, 21–25), parameters of liver function (15, 18, 20), renal replacement therapy (15), and comedication such as rifampin (a potent P-glycoprotein inducer) (7), have already been described. However, the effects of other possible covariates, such as the presence of acute respiratory distress syndrome (ARDS), peritonitis, and single nucleotide polymorphisms of the P glycoprotein, remain unclear.

Describing and quantifying the effects of such covariates would

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enable physicians to identify patient subgroups at high risk for therapy failure and could be the basis for a simple dose adjustment as well as for Bayesian-based therapeutic drug monitoring (26). The aim of this study was to assess important covariates of the pharmacokinetics of linezolid and the resulting serum concentrations in critically ill patients.

MATERIALS AND METHODS

Patients. Medical-surgical critically ill patients hospitalized in three ICUs within the Department of Anesthesiology, University Hospital of Munich, Munich, Germany, were included in this study. The presented data originated from 30 study patients described recently (13) and 22 consecutively included patients (in total, 52 patients) who were treated with linezolid (patient group 1). As an independent patient group for simulations, patients from the same study who were not treated with linezolid but were treated with meropenem, piperacillin-tazobactam, cefepime, or ciprofloxacin (134 patients) were chosen (patient group 2). A main inclusion criterion was a clinically suspected or confirmed infection. Detailed inclusion and exclusion criteria were described previously (13) (see https://clinicaltrials.gov/ct2/show/NCT01793012). Written informed consent was obtained from all study patients or their legal representatives.

Study design. The study protocol of this monocentric prospective observational study was approved by the Institutional Review Board and carried out according to the principles of the Declaration of Helsinki. Study patients received 600 mg linezolid b.i.d. by short-duration intravenous infusions or orally. Before day 1 (beginning of the study), patients had already received 0 to 4 linezolid administrations. Blood samples were obtained at multiple time points over 4 days for determination of linezolid concentrations (see Fig. S1 in the supplemental material). Medical staff recorded the exact time of blood sampling. After immediate delivery to the Institute of Laboratory Medicine, University of Munich, samples were centrifuged, and serum was stored at −80°C. Linezolid concentrations were determined from serum samples by a previously described liquid chromatography-tandem mass spectrometry (LC-MS/MS) method (27). Validation revealed good analytical performance, with inaccuracy of <6% and imprecision of <7.3% (coefficient of variation [CV]) for six quality control samples (0.38 to 16.0 mg/liter). This method was found to be linear over the range of measured linezolid values. Numerous clinical and laboratory data were determined by routine methods once daily (see Table S1 in the supplemental material). The indocyanine green elimination rate (LiMon technology; Pulsion Medical Systems, Feldkirchen, Germany) to monitor liver perfusion and P-glycoprotein single nucleotide polymorphisms were determined once per patient. Creatinine concentrations were determined once daily from serum and urine samples collected over 24 h, and creatinine clearance rates were calculated as described previously (13).

Basic population pharmacokinetic analysis. Based on linezolid serum measurements from patient group 1, a population pharmacokinetic nonlinear mixed-effects model was built by using NONMEM 7.3.0 (Icon Development Solutions, Ellicott City, MD, USA). The first-order conditional estimation method with interaction was used for parameter estimation. All modeling processes were aided by Perl-speaks-NONMEM (28), XPOSE 4.5.0 (29), MATLAB R2015a (The MathWorks, Inc., Natick, MA, USA), and R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). The structural pharmacokinetic model was built empirically, starting with a one-compartment model with linear elimination kinetics. Up to three compartments; linear and Michaelis-Menten elimination kinetics; and additive, proportional, and combined error models were evaluated. Interindividual variability (IIV) terms with and without covariance were tested on all pharmacokinetic parameters. The final structural model with IIV terms is called the basic population pharmacokinetic model.

A P value of ≤0.01 was considered statistically significant. All P values were calculated based on a chi-square distribution of objective function values. Each step was evaluated in means of the drop in the objective function value and goodness-of-fit plots. A bootstrap statistic with 1,000 samples was calculated for the final model.

Strategies for identifying covariates. The procedure for identifying influencing covariates is shown in Fig. 1A to C. Thirty covariate candidates defined based on clinical reasoning were analyzed by using an explorative univariate analysis for patient group 1 (Fig. 1A). Exclusion of the parameters without any significant correlation to linezolid concentrations led to a refined set of covariate candidates. The resulting covariate candidates were analyzed independently by using a multivariate analysis (Fig. 1B) and a population pharmacokinetic model (Fig. 1C). IBM SPSS statistics 23 (International Business Machines Corporation [IBM], Armonk, NY, USA) was used for the univariate and multivariate analyses, while the population pharmacokinetic analysis was done by using software mentioned above for the basic population pharmacokinetic analysis.

In the univariate analysis, the Mann-Whitney U test for categorical covariates and Spearman correlation for continuous covariates with a P value of <0.01 were used. The relationships between linezolid concentrations (values at 1 h postdose and trough values) and covariate values were investigated. Similarly, relationships between liver parameters (antithrombin and factor V) and the presence or absence of peritonitis and between lactate concentrations and the "cardiovascular" sequential organ failure assessment (SOFA) subscore (30) were determined. In the multivariate analysis (linear regression model with backward elimination), we considered a P value of <0.0016, which was derived from Bonferroni correction, to account for multiple comparisons. In the population pharmacokinetic model, stepwise covariate modeling (SCM) (forward inclusion criterion of a P value <0.05 and backward elimination criterion of a P value of <0.01) was used to assess the covariate candidates for pharmacokinetic parameters. All covariate values were normalized to their respective population medians. For the population pharmacokinetic model, laboratory parameters were allowed to be time varying, with one change per day.

Strategy for quantifying covariate effects. The effect sizes of covariates on the area under the concentration-time curve over 12 h (AUC_{12}) were determined (Fig. 1D and E). For the covariates identified in the multivariate analysis, we compared AUC_{12} values (first versus fourth quartiles of continuous covariates and presence or absence of categorical covariates) as predicted by the basic population pharmacokinetic model (Fig. 1D). AUC_{12} values from the covariate population pharmacokinetic model (at linezolid treatment days 1 and 4) were computed to explore the influence of covariates identified in the population pharmacokinetic analysis (Fig. 1E). To cover a broader range of realistic covariate value constellations, 67,000 patients with covariate values as observed in the larger patient group 2 (each patient simulated 500-fold) were used, and short-duration infusions (60 min) of 600 mg linezolid every 12 h were simulated.

Assessment of lower threshold for target concentration range. The threshold for antimicrobial efficacy was defined as an AUC_{12} of >100 mg · h/liter. This is based on the results of a large compassionate-use study showing that a higher rate of clinical success is reached when the ratio of the AUC_{24} to the MIC of the causative strain is >80 to 120 (6). Therefore, we considered an AUC_{24}/MIC ratio of >100 to be a relevant pharmacokinetic/pharmacodynamic parameter in accordance with data reported in the literature (8, 15). A concentration of 2 mg/liter, which inhibits >90% of relevant causative strains (31, 32), was used as the MIC. Using this MIC, improved efficacy can be assumed at an AUC_{24} of >200 mg · h/liter. As we measured linezolid concentrations over only 12 h at days 2 to 4, we defined the target as an AUC_{12} of >100 mg · h/liter.

RESULTS

Patient group characteristics and observed linezolid exposure. Several linezolid plasma measurements per patient (median, 32) were analyzed. All patients received intravenous short-duration infusions (10 to 120 min) or oral administrations (4 patients; 11 oral administrations in total). A high level of heterogeneity of characteristics was observed for both patient groups (Table 1). The most frequent causes of severe infections in group 1 were

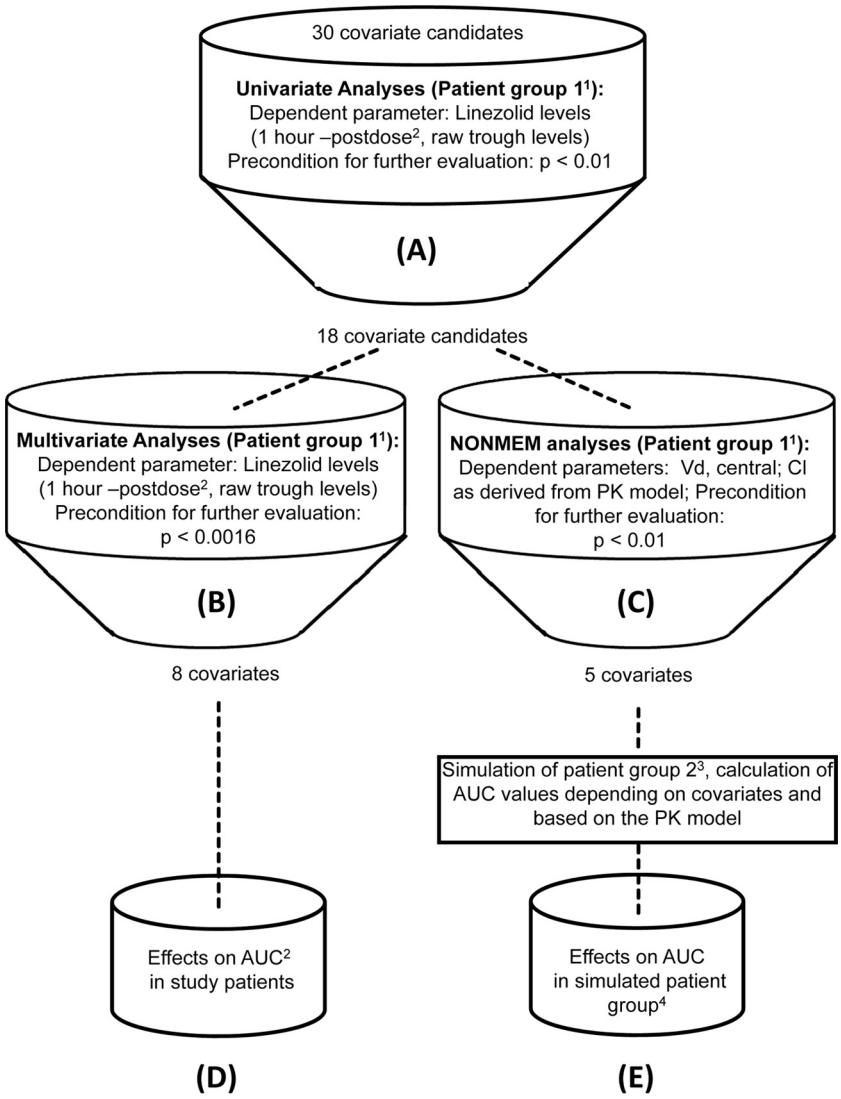

FIG 1 Strategy for identifying covariates that influence linezolid concentrations. AUC, area under the linezolid concentration curve; Vd, volume of distribution for linezolid; Cl, linezolid clearance; PK, pharmacokinetic. [1], study patients (for characteristics, see Table 1); [2], predicted AUC values from the basic population pharmacokinetic model; [3], independent patient group of 134 patients (for characteristics, see Table 1); [4], simulated patient group (67,000 patients) with 500-fold simulation of all patients from group 2.

pneumonia (67%) followed by peritonitis (17%). Patients showed high variabilities of weight (range, 44 to 120 kg), severity scores (Acute Physiology and Chronic Health Evaluation II [APACHE II] score range, 9.0 to 38), renal function (estimated creatinine clearance rate range, 5 to 293 ml/min), and liver-related parameters (e.g., total bilirubin level range, 3.4 to 739 μmol/liter). Patient characteristics for patient group 2 were similar, with no significant differences ($P > 0.05$) between both patient groups for all parameters shown (Table 1). We observed a high percentage of AUC values below the target range in this enlarged patient group (64% on both study days 1 and 4). Median AUC_{12} values (interquartile ranges) of linezolid in the 52 patients were 72.8 mg · h/liter (47.4 to 126.7 mg · h/liter) on day 1 and 74.6 mg · h/liter (48.8 to 115.1 mg · h/liter) on day 4.

Explorative univariate analysis. The univariate explorative analysis revealed 18 different parameters that correlated significantly ($P < 0.01$) with either trough values or linezolid blood concentrations at 1 h postdose (see Table S1 in the supplemental material). These included parameters from the parameter groups "patient demographics," "liver," "kidney," "acid-base balance," "disease," "inflammation," and "specific treatments." In contrast, no significant correlations were observed for parameters from the patient groups "severity of disease," "genetics of P-glycoprotein," and "protein."

Multivariate analysis. In the multivariate analysis, 8 covariate candidates correlated independently and significantly ($P < 0.0016$) with either linezolid trough values or values at 1 h postdose (see Table S2 in the supplemental material). These were two parameters from the parameter group liver (antithrombin and fibrinogen), two parameters from the parameter group patient size (height and weight) as well as C-reactive protein (CRP) level, creatinine clearance rate, presence or absence of ARDS, and lactate level. Lactate correlated with the cardiovascular SOFA subscore ($P < 0.01$ on days 1 and 4).

TABLE 1 Clinical and demographic characteristics of study patients and the independent patient group

Parameter[c]	Value for patient group											
	1[a]						2[b]					
			Quartile						Quartile			
	No. (%)	Min	1st	2nd	3rd	Max	No. (%)	Min	1st	2nd	3rd	Max
Total study patients	52 (100)						134 (100)					
Male patients	33 (63)						82 (61)					
Age (yr)		28	49	58	63	84		22	49	58	67	94
Body wt (kg)		44	65	76	91	120		40	65	74	85	150
Body ht (cm)		158	168	174	180	196		150	166	172	180	198
Patients with CRRT	16 (31)						32 (24)					
Patients after liver TX	7 (13)						20 (15)					
Patients after lung TX	15 (29)						31 (23)					
APACHE II score on day 1		9.0	22.5	28.0	33	38		6.0	20.0	26.5	32.8	51
SOFA score on day 1		2	10	11	14	21		2	9	12	14	23
Patients with ARDS	15 (29)						28 (21)					
Patients with peritonitis	9 (17)						20 (15)					
Patients with pneumonia	35 (67)						80 (60)					
Cr Cl rate (ml/min) on day 1		5	53	81	109	293		2	29	66	106	251
Total bilirubin level (μmol/liter) on day 1		3.4	10.3	17.1	47.9	739		3.4	10.3	18.8	54.7	426
Fibrinogen level (μmol/liter) on day 1		2.6	10.1	13.0	15.4	24.4		2.4	9.4	12.8	16.1	27.3
Antithrombin level (%) on day 1		32	65	82	91	125		21	58	75	88	130
Lactate level (mmol/liter) on day 1		0.53	1.35	1.91	2.87	10.7		0.55	1.1	1.5	2.05	28.3
CRP level (mg/liter) on day 1		11	69	126	184	376		5	76	122	214	468

[a] Fifty-two study patients receiving linezolid.
[b] Independent patient group of 134 patients needed to create the simulated patient group (Fig. 1).
[c] CRRT, continuous renal replacement therapy; TX, transplantation (within 28 days before the beginning of the study); Cr Cl, creatinine clearance of noncontinuous renal replacement therapy patients.

Population pharmacokinetic models. Two compartments with first-order elimination and absorption with complete oral bioavailability and a combined error model (proportional and additive component) were found to be most suitable to describe the data in the basic population pharmacokinetic model. The bootstrap population estimates of the median central volume of distribution (V_c) and peripheral volume of distribution (V_p) were 15 liters (95% confidence interval, 8.58 to 20.67 liters) and 26.55 liters (95% confidence interval, 21.24 to 32.63 liters), respectively, with a median elimination clearance (CL) rate of 7.92 liters/h (95% confidence interval, 6.27 to 9.74 liters/h), a median intercompartmental clearance rate of 65.59 liters/h (95% confidence interval, 45.92 to 109.61 liters/h), and a median absorption rate constant of 1.72 h^{-1} (95% confidence interval, 0.66 to 2.38 h^{-1}). Interindividual variability terms were kept for V_c (37%) and CL (58%) (coefficients of variation).

Covariates in the covariate population pharmacokinetic model were body weight and peritonitis on V_c and fibrinogen, lactate, and ARDS on CL (see Table S3 in the supplemental material). Positive correlations between body weight and V_c, fibrinogen, and CL and a negative correlation between lactate and CL were determined. V_c was increased by a median of 53% (95% confidence interval, 16% to 111%) for patients with peritonitis, while CL was increased by a median of 82% (95% confidence interval, 26% to 162%) for patients with ARDS. Covariate inclusion reduced the observed remaining (unexplained) interindividual variability by 52% for V_c and 28% for CL. The resulting individual V_c and CL values were calculated as follows, with θ representing the respective population estimates and η representing the interindividual variability incorporating covariates normalized to their median: $V_c = \theta_V c \cdot e^{\eta_1} \cdot \text{weight}_{\text{norm.}}^{1.31} \cdot 1.53$ (if peritonitis) and $\text{CL} = \theta_{\text{CL}} \cdot e^{\eta_2} \cdot \text{fibrinogen}_{\text{norm.}}^{0.04} \cdot \text{lactate}_{\text{norm.}}^{-0.21} \cdot 1.82$ (if ARDS). For final goodness-of-fit plots, see Fig. S2 in the supplemental material.

Covariate effects on AUC_{12} in patient group 1. We observed substantially lower median AUC_{12} values for patients with high height, fibrinogen, antithrombin, CRP, and creatinine clearance values than for patients with low values (medians of 34 to 68% for the fourth quartile versus the first quartile of each parameter) (Fig. 2). Patients with ARDS also had lower median AUC_{12} values (56% on day 1). In contrast, higher median values were observed for patients with high lactate concentrations (224% on day 1 for the fourth quartile versus the first quartile).

Covariate effects on AUC_{12} in the simulated patient group. The target attainment frequency (AUC_{12} of >100 mg · h/liter) for the simulated patients within the first and fourth quartiles of

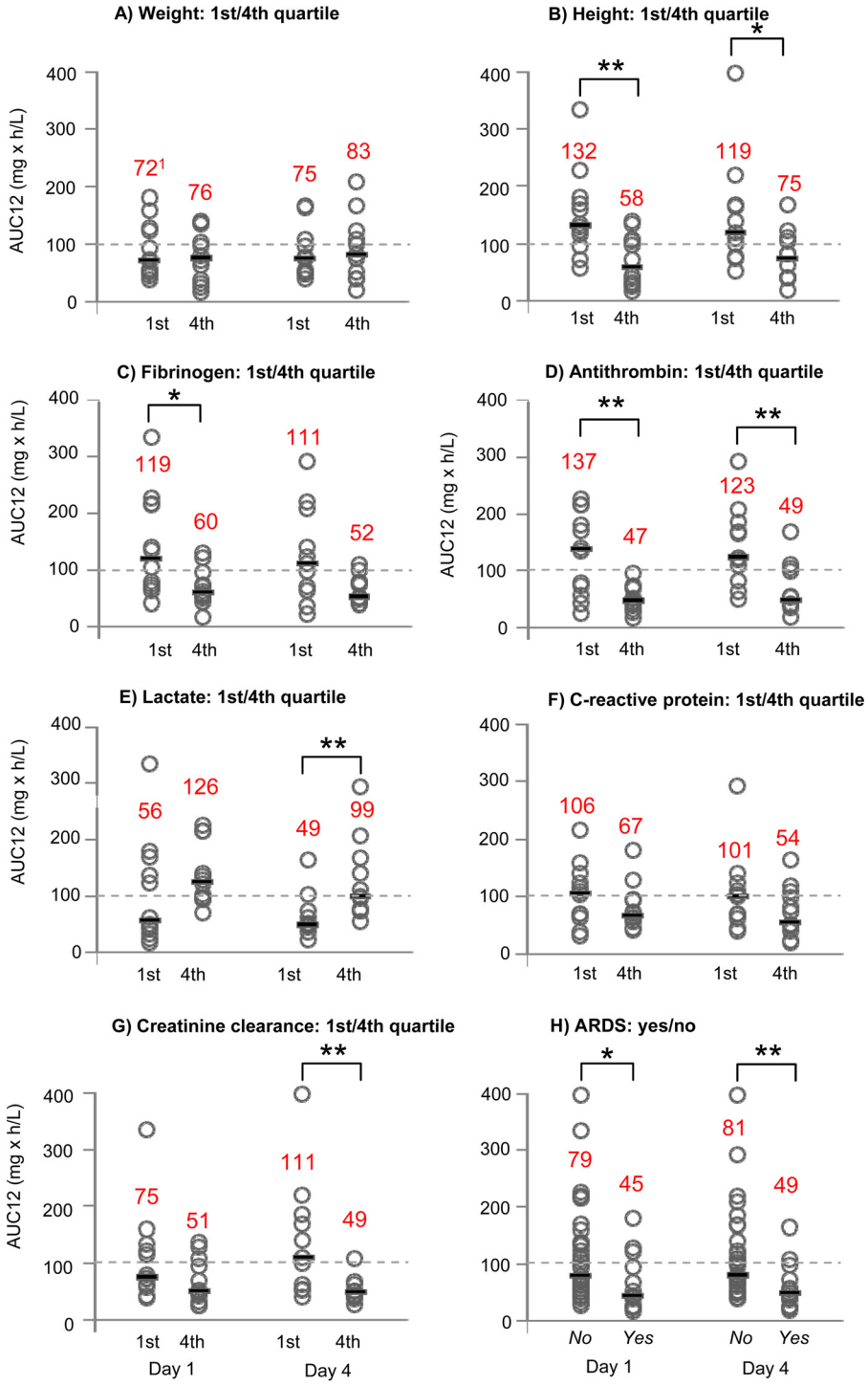

FIG 2 Covariates in study patients (patient group 1). [1], medians of AUC_{12} are presented in red. *, $P < 0.05$; **, $P < 0.01$. 1st indicates the lowest quartile of concentrations of parameters shown in panels A to G, 4th indicates the highest quartile of concentrations of parameters shown in panels A to G. The dashed line indicates the lower threshold for the target range.

weight, fibrinogen levels, and lactate levels differed by ≤9% for each respective pair on days 1 and 4 (Fig. 3). In contrast, there was a substantially greater difference in target attainment between patients with and those without ARDS (relative differences of 16% on day 1 and 23% on day 4). Patients with ARDS only rarely reached the target AUC_{12} (≤6% on days 1 and 4). Despite the higher volume of distribution in patients with peritonitis, these patients exhibited higher linezolid AUC_{12} values. Concentrations of factor V and antithrombin were lower in patients with peritonitis ($P < 0.01$ on all 4 study days) in patient groups 1 and 2.

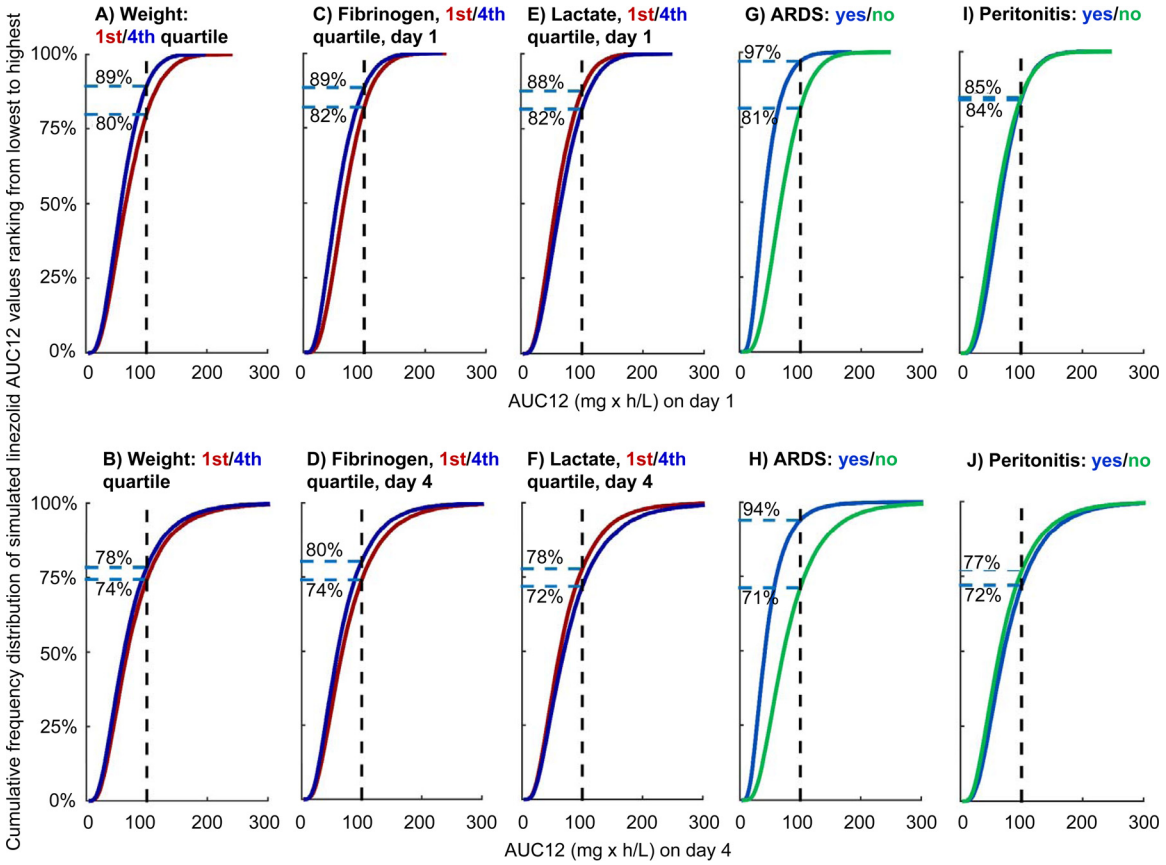

FIG 3 Percentage of simulated linezolid AUC_{12} values expected for the simulated patient group, ranked from lowest to highest. Shown are effects of covariates in simulated patients. Cumulative frequency distributions of AUC_{12} values are presented for the specified values of respective covariates.

DISCUSSION

The approach of combining a multivariate analysis and a population pharmacokinetic analysis led to a number of previously unknown factors related to insufficient concentrations of linezolid when administered at standard doses. Beyond the important identification of such risk factors, the population pharmacokinetic simulations provide information on the magnitude of the respective differences in linezolid exposure and may thus be used for dose adjustments. Furthermore, it allows prediction of covariate effects in large populations such as our simulated patient group. In this group, the presence of ARDS had the greatest effect on linezolid clearance, leading to potentially subtherapeutic linezolid concentrations in almost every patient (≥94%). Other predictors of insufficient linezolid concentrations included low lactate concentrations, high fibrinogen concentrations, and high weight. In contrast to ARDS, these risk factors had minor to moderate effects, with the target attainment probability differing between the respective highest and lowest quartiles by ≤9%. The probability of insufficient concentrations might therefore be increased, especially in patients with combined alterations of these covariates. Some risk factors, such as a low creatinine clearance rate, were identified by the multivariate analysis but not by the population pharmacokinetic approach, which may be attributed to greater demands pertaining to the data quality for mixed-effects modeling. The approach of combining two different analyses therefore led to the identification of additional risk factors. While covariates are indicators but not necessarily causes of changes in linezolid pharmacokinetics, previously reported data suggest plausible causal links via pathophysiological processes in most cases (Fig. 4).

Surprisingly, the covariate with the strongest influence on simulated linezolid blood concentrations was ARDS, related to an increase in linezolid clearance by 82%. It is tempting to speculate that the reason for this phenomenon might be the large amount of reactive oxygen species in the lungs of ARDS patients (33), which may oxidize linezolid nonenzymatically. The observed subtherapeutic linezolid concentrations, especially in ARDS patients, may pose a severe threat to critically ill patients. To the best of our knowledge, there are no studies evaluating the outcome for ARDS patients with infections treated by linezolid.

A second interesting covariate was lactate. Higher lactate concentrations were associated with lower linezolid clearance rates and higher linezolid concentrations. To further evaluate whether lactate might at least in part reflect the status of the patient's cardiac output, we investigated the correlation of lactate concentrations with the cardiovascular SOFA subscore on the same day, revealing a significant positive correlation on different study days. A possible reason for the decreased linezolid clearance in the case of decreased cardiac output might thus be reduced renal excretion and reduced linezolid metabolism. Indeed, increased cardiac output has been assumed to increase antibiotic clearance in general (34).

In contrast to the presence of ARDS and lactate, the cofactors weight, creatinine clearance, and impairment of liver function

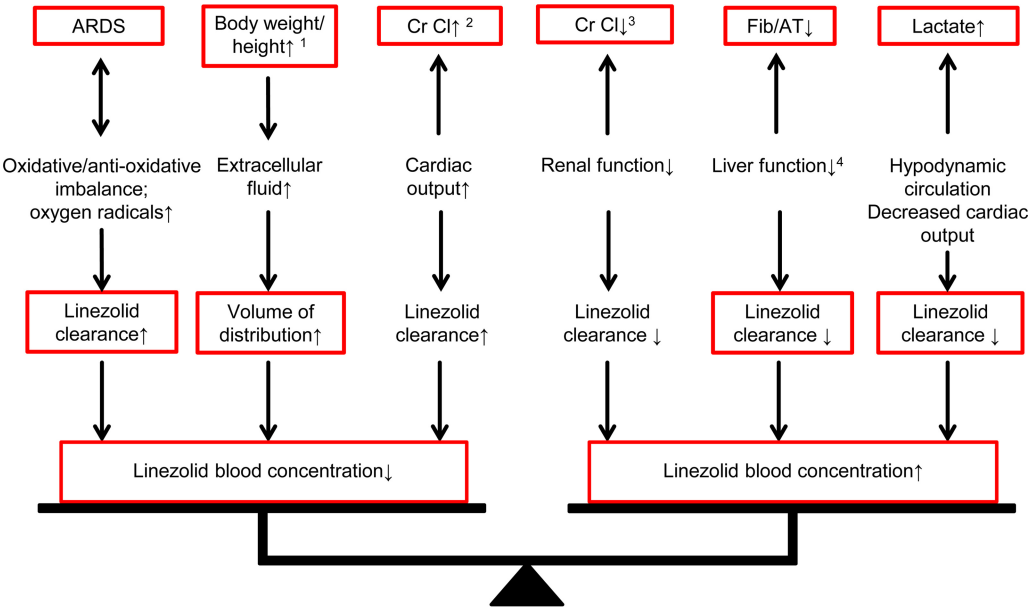

FIG 4 Proposed model for cofactors influencing linezolid pharmacokinetics. Analyses in this study are indicated in red. Cr Cl, creatinine clearance; Fib, fibrinogen; AT, antithrombin. [1], also described in references 18 and 21–25; [2], described in reference 14; [3], described in references 18, 19, and 20–22; [4], described in references 18, 20, and 15.

were described previously (15, 18–25) (Fig. 4). As the liver enzymes metabolizing linezolid are still unknown, the exact role of the liver in linezolid metabolism is not clear (20). However, the fact that different studies described a correlation between impaired liver function and increased linezolid clearance and that hepatocyte microsomes have been shown to be able to metabolize linezolid (11) as well as the results from our analyses (e.g., see Table S1 in the supplemental material) argue for an involvement of the liver in linezolid metabolism.

Furthermore, in the population pharmacokinetic analysis, the presence of peritonitis was associated with an increase of the central volume of distribution of linezolid by about 50%. The fact that linezolid concentrations apparently did not decrease in either the simulated patient group (Fig. 3) or patient group 1 (data not shown) may be explained by the coincidence of peritonitis with impaired liver function in our patients: concentrations of factor V and antithrombin were significantly lower in patients with peritonitis.

Finally, CRP was negatively associated with linezolid concentrations. However, this association might rather be a consequence of therapy with therapeutic linezolid concentrations resulting in better bacterial killing and a faster decrease of CRP concentrations.

This study has some limitations. By choosing a heterogeneous patient group, we tried to ensure coverage of a large and clinically meaningful range of potential covariates. Nevertheless, the limited number of subjects clearly cannot represent all relevant patient groups and, in conjunction with the high observed variability, leads to some statistical limitations. Therefore, we probably could not identify all relevant cofactors. Additionally, we did not evaluate proper outcome parameters. The influence of the identified cofactors on therapeutic outcome therefore could not be determined. Finally, we did not measure free linezolid concentrations, which probably could be more informative since only free linezolid has an antibiotic effect. However, protein binding of linezolid is only about 31% (9), and the target threshold used was selected in accordance with previously reported data where total linezolid concentrations were also determined (6).

In conclusion, 600 mg linezolid b.i.d. is often potentially insufficient in critically ill patients, which was shown recently (13). In this study, a number of additional indicators of insufficient exposure were identified. ARDS was the most important one, leading to potentially subtherapeutic concentrations in almost every simulated patient. In order to increase the probability of successful treatments, we propose further studies on different dosing regimens such as prolonged infusions and the evaluation of the effect of therapeutic drug monitoring on outcome in critically ill patients. Prospective studies investigating the influence of cofactors on therapeutic outcomes may be useful to evaluate the clinical relevance of the reported pharmacokinetic relationships.

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We state that there is no conflict of interest relevant to the study.

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